Prevention of Digitoxin Poisoning by Various Steroids

HANS SELYE, JAN JELINEK, and MILOSLAV KRAJNY

Abstract □ Experiments on rats indicate that spironolactone as well as several anabolic androgens protect the rat against an otherwise fatal digitoxin poisoning, whereas numerous other hormonally active or inactive steroids are ineffective in this respect. Among those tested, all potent anabolic androgens possess antidigitoxin activity; however, male hormonal actions do not appear to be indispensable for this protective effect since spironolactone is devoid of anabolic and androgenic potency, yet highly active in antagonizing digitoxin.

Keyphrases □ Digitoxin poisoning—steroid effect □ Androgenic steroids—antidigitoxin activity □ Spironolactone—antidigitoxin activity □ Anabolic steroids—antidigitoxin activity

The observation that overdosage with pure mineralocorticoids can produce hypertension, cardiac failure, and severe dropsy in the chick (1) and rat (2) was followed by clinical investigations showing increased urinary mineralocorticoid excretion in patients with certain types of hypertension, nephrosis, cardiac failure, or toxemia of pregnancy (3–5), and eventually, the responsible factor could be isolated and identified as aldosterone (6, 7). Hence, an intensive search began for a compound that might block the actions of mineralocorticoids and these investigations culminated in the discovery of spironolactone (8, 9), a hormonally inactive steroid which, according to current opinion, antagonizes both aldosterone and desoxycorticosterone by a selective competitive inhibition of the mineralocorticoid effect at the level of the renal tubule. However, it soon became evident that the compound also possesses apparently quite unrelated activities. For example, it offers protection against the cardiovascular lesions produced by dihydrotachysterol (DHT) overdosage (10) and the same is true of a closely related antimineralocorticoid "SC-11927" (11). More recent studies have shown that spironolactone also protects the rat against digitoxin poisoning (12) as well as against the sedative and anesthetic effects of various steroid and nonsteroid compounds (13).

Independently of these studies it had been noted that not only the body-weight loss but even the soft-tissue calcifications elicited by DHT overdosage are inhibited by simultaneous treatment with various anabolic steroids such as methyltestosterone (14–17) or 17-ethyl-19-nortestosterone (18). Extensive studies with a large number of androstane derivatives have subsequently shown that the anti-DHT effect is more closely related to the anabolic than to the androgenic potency; norbolethone, fluoxymesterone, and other predominantly anabolic compounds with comparatively weak androgenic potency are the most potent antagonists of DHT (19).

Finally, it had been found that spironolactone also antagonizes the adrenal necrosis normally produced in rats by dimethylbenzanthracene (20).

All these observations clearly indicated that contrary to current opinion not all actions of spironolactone are merely the consequences of a mineralocorticoid-blocking effect. Its ability to protect against DHT, various anesthetics, and digitoxin is shared by predominantly anabolic androgens but does not seem to be inseparably linked to anabolic potency or any other known steroid-hormone action since spironolactone itself is virtually devoid of androgenic, anabolic, estrogenic, luteoid, corticoid, or anesthetic actions. The purpose of the experiments to be described here was to compare the possible protective effect of numerous hormonal and nonhormonal steroids, potassium-sparing agents, and potassium chloride in rats subjected to fatal acute digitoxin poisoning under standard conditions.

MATERIALS AND METHODS

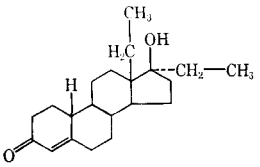
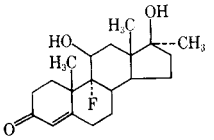
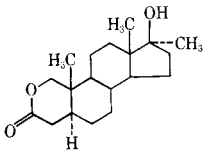
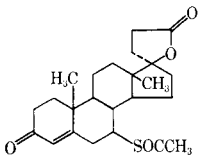
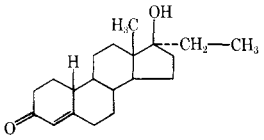
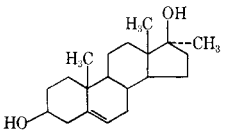
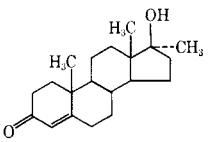
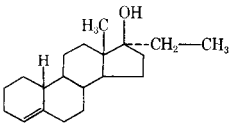
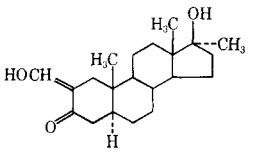
Two-hundred and fifty female rats of the Holtzman Farms (Madison, Wis.) with a mean initial body weight of 100 g. (range 90–110 g.) were divided into 25 equal groups and treated as outlined in Table I. Digitoxin (1 mg./100 g. body weight) was administered in 1 ml. of water once daily by stomach tube to all rats. The animals of Group 1 acted as controls and received no other medication, whereas those of Groups 2–25 were pretreated with various potentially prophylactic agents.

The steroids spironolactone, norethandrolone, oxandrolone, norethynodrel (Searle), testosterone, methylandrostenediol, desoxycorticosterone acetate, progesterone (Ciba), ethylestrenol, nandrolone phenpropionate, nandrolone decanoate (Organon), fluoxymesterone (Upjohn), oxymetholone (Parke-Davis), ethisterone (Frosst), acetoxypregnenolone (Chemical Specialities), methyltestosterone, estradiol, cortisol acetate, pregnanedione, pregnenolone (Schering), and norbolethone (Wyeth), were all administered twice daily at the dose of 10 mg. in 1 ml. of water by stomach tube, beginning on the fifth day prior to the initiation of digitalization. This comparatively long pretreatment was given because preliminary experiments had shown that the antidigitoxin effect of the active steroids takes 2–5 days to reach its full development.

The individual dose of KCl was 1 mmole,, that of amiloride·HCl (Merck Sharp & Dohme) 300 mcg., and that of triamterene (Smith Kline & French) 2 mg., always in 1 ml. of water administered twice daily by stomach tube. In order to avoid complications owing to the hyperkalemia elicited by the last-mentioned three compounds and, because of their more rapid action, treatment with these nonsteroidal agents was started 48 hr. before administration of digitoxin. The doses of KCl and of the potassium-sparing agents were selected, on the basis of preliminary experiments, to produce the greatest potassium flooding of the tissues compatible with survival.

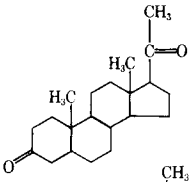
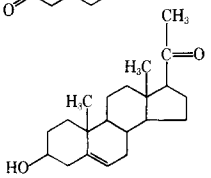
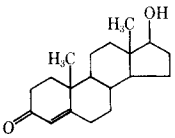
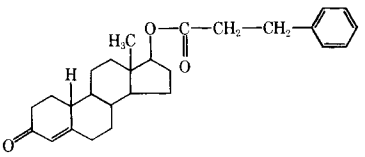
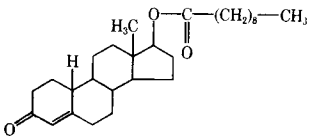
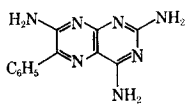
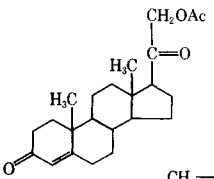
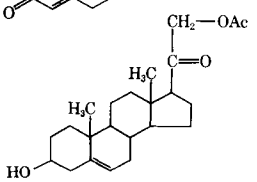
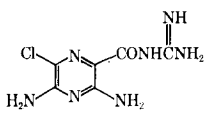
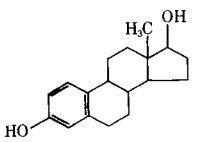
Throughout the experiment, the rats were maintained exclusively on a special preparation (Purina Laboratory Chow, Ralston Purina Co. of Canada) and tap water. The convulsive motor disturbances typical of digitoxin poisoning were assessed in terms of an arbitrary scale in which 0 = no change, + = uncertain gait, ++ = spastic contractions of the skeletal muscles but the animal maintains up-

Table I—Prevention of Digitoxin Poisoning by Various Steroids

Group	Treatment[a]		Mort., %	Final Body Weight, g.[b]	Preputial Gland Weight, mg.[b]
1	Control	None	70	76 ± 5	30 ± 2
2		Norbolethone: *dl*-13-ethyl-17-hydroxy-18, 19-dinor-17α-pregn-4-en-3-one	0	130 ± 2***	130 ± 8***
3		Fluoxymesterone: 17α-methyl-9α-fluoro-11β, 17β-dihydroxy-androst-4-ene-3-one	0	130 ± 1***	153 ± 11***
4		Oxandrolone: 17α-methyl-17β-hydroxy-2-oxa-5α-androstan-3-one	0	121 ± 3***	117 ± 13***
5		Spironolactone: 17-hydroxy-7-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid γ-lactone	0	120 ± 4***	55 ± 6 N.S.
6		Norethandrolone: 17α-ethyl-17β-hydroxy-19-norandrost-4-ene-3-one	10	116 ± 4***	105 ± 5***
7		Methylandrostenediol: 17α-methyl-3β,17β-dihydroxy-androst-5-ene	0	111 ± 2***	170 ± 13***
8		Methyltestosterone: 17α-methyl-17β-hydroxy-androst-4-en-3-one	10	108 ± 4***	137 ± 12***
9		Ethylestrenol: 17α-ethyl-17β-hydroxy-estr-4-ene	0	101 ± 3**	127 ± 8***
10		Oxymetholone: 17α-methyl-17β-hydroxy-2-(hydroxymethylene)-5α-androstan-3-one	40	95 ± 3 N.S.	120 ± 11***
11	KCl	Potassium chloride	70	89 ± 1 N.S.	33 ± 4 N.S.

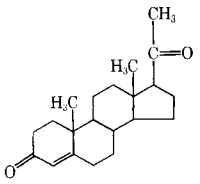
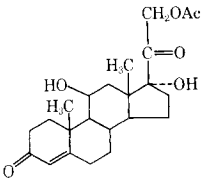
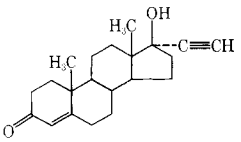
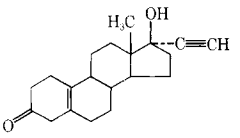
(*continued on next page*)

Table I—*(continued)*

Group	Treatment[a]	Mort., %	Final Body Weight, g.[b]	Preputial Gland Weight, mg.[b]
12	 Pregnanedione: pregnane-3,20-dione	50	89 ± 7 N.S.	71 ± 8***
13	 Pregnenolone: 3β-hydroxy-pregn-5-ene-20-one	60	89 ± 4 N.S.	71 ± 7***
14	 Testosterone: 17β-hydroxy-androst-4-ene-3-one	60	87 ± 4 N.S.	71 ± 4***
15	 Nandrolone phenpropionate: 17β-hydroxyestr-4-en-3-one 17-phenylpropionate	60	84 ± 1 N.S.	46 ± 12 N.S.
16	 Nandrolone decanoate: 17β-hydroxyestr-4-en-3-one 17-decanoate	50	83 ± 10 N.S.	91 ± 7***
17	 Triamterene: 2,4,7-triamino-6-phenyl-pteridine	100	82 ± 6 N.S.	45 ± 4 N.S.
18	 Desoxycorticosterone acetate: 21-hydroxypregn-4-ene-3,-20-dione 21 acetate	60	82 ± 2 N.S.	40 ± 4 N.S.
19	 Acetoxypregnenolone: 3β,21-dihydroxy-pregn-5-ene-20-one 21 acetate	80	80 ± 3 N.S.	39 ± 3 N.S.
20	 Amiloride: *N*-amidino-3,5-diamino-6-chloropyrazinecarboxamide	100	78 ± 2 N.S.	31 ± 3 N.S.
21	 Estradiol: 3β,17β-dihydroxy-estra-1,3,5-triene	100	75 ± 1 N.S.	39 ± 4 N.S.

(continued on next page)

Table I—(*continued*)

Group	Treatment[a]	Mort., %	Final Body Weight, g.[b]	Preputial Gland Weight, mg.[b]
22	 Progesterone: pregn-4-ene-3,20-dione	40	74 ± 3 N.S.	46 ± 5 N.S.
23	 Cortisol acetate: 11β,17α,21-trihydroxy-pregn-4-ene-3,20-dione	80	64 ± 2 N.S.	37 ± 3 N.S.
24	 Ethisterone: 17α-ethynyl-17β-hydroxy-4-androsten-3-one	100	—	40 ± 2 N.S.
25	 Norethynodrel: 17-hydroxy-19-nor-17α-pregn-5(10)-en-20-yn-3-one	100	—	35 ± 3 N.S.

[a] In addition to the compounds listed in this column the animals of all groups were given digitoxin as described in the text. [b] The significance of the difference between control values (Group 1) and the treated animals (Groups 2–25) is expressed as follows: ** = $p < 0.005$, *** = $p < 0.001$, N.S. = not significant.

right position, and +++ = intense convulsions with the animal lying on its side. All animals were killed on the sixth day and the percentage mortality rate, listed in Table I, corresponds to the animals that succumbed by that time. The first manifestations of motor disturbances became evident within 24 hrs. after initiation of digitalization and became progressively worse in the rats that lost body weight. Thus, on the last day of the experiment, in the inadequately protected groups, even the survivors showed evidence of severe digitoxin poisoning. In order to permit comparisons between the anabolic (or anticatabolic), androgenic, and antidigitoxin effects, the final body weight and the preputial-gland weight was determined in all animals that survived to within 24 hr. of terminating the experiment and the means of these determinations (with standard errors) are also listed in the table.

RESULTS

In Table I the groups are arranged in the order of decreasing final body weights. It will be noted that, under the prevailing experimental conditions, digitoxin produced 70% mortality and a considerable loss of body weight from the initial mean value of 100 g. in otherwise untreated controls (Group 1); all survivors were in continuous severe convulsions and evidently moribund at the end of the experiment.

By contrast, the rats treated with spironolactone or the most strongly anabolic steroids (Groups 2–6) showed no mortality and no convulsions. These anabolic steroids also prevented the body-weight loss otherwise induced by heavy digitoxin poisoning, but this was true also of spironolactone which has no inherent anabolic activity; it presumably prevented loss of body weight only by counteracting the anorectic and catabolic effect of severe digitoxin poisoning. All these anabolic steroids also exerted androgenic effects as judged by the enlargement of the preputial glands but, as expected, spironolactone exhibited no virilizing potency. Less active anabolic steroids (Groups 7–10) also offered definite protection against digitoxin-induced mortality which was also associated with stimulation of the preputial glands. All remaining compounds (Groups 11–25) were virtually devoid of any significant protective effect against mortality or body-weight loss and, correspondingly, showed little or no preputial gland-stimulating potency.

DISCUSSION

Since spironolactone is a potent potassium-sparing agent, and digitalis intoxication in man is counteracted by potassium, it might have been suspected that the protective effect of the antimineralocorticoid is merely due to its potassium-retaining effect. However, here, even near toxic doses of KCl, or treatment with other highly potent potassium-sparing agents such as amiloride and triamterene, offered no protection against the large doses of digitoxin used. It is evident, furthermore, that several steroids other than spironolactone can protect the rat against fatal digitoxin intoxication irrespective of their effects upon electrolyte metabolism.

On the other hand, there exists a striking parallelism between the antidigitoxin and the anticatabolic-androgenic effect as determined by earlier work in which these same steroids were comparatively assayed for their ability to prevent loss of body weight and stimulate the preputial glands in rats chronically treated with dihydrotachysterol (19). In the present experiments, acute intoxication with digitoxin may have modified the anticatabolic effect and the response of the preputial glands. In addition, the period of steroid treatment was short. Hence, the results do not correspond in every detail to those of the earlier work just cited; yet, in general, the outcome is the same in that the most potent antidigitoxin compounds are also most powerful in preventing body-weight loss and (with the exception of spironolactone) in stimulating the preputial glands.

Although in the table, the potentially protective compounds are listed in decreasing order of their anticatabolic action, the exact position of a compound in this order is largely accidental. Differences of a few grams may depend upon the amount of food that happens to be in the gastrointestinal tract at the time of weighing. Still, the rats in Groups 2–9, which were undoubtedly best protected

against digitoxin-induced mortality, also showed the highest final body weights and (with the exception of spironolactone) the highest preputial-gland weights.

In interpreting these data it must be remembered that, if a compound prevents digitoxin poisoning, it will naturally inhibit not only the mortality but also the loss of body weight. Hence, in themselves, these experiments would not prove a causal relationship between anticatabolic and antidigitoxin actions but earlier observations have shown these same compounds to possess anabolic potencies in other tests (19). Similarly, this experimental arrangement being chosen to demonstrate antidigitoxin activity, was not particularly suitable for the accurate assessment of androgenic potency (which is, in any event, not optimally reflected by the weight of the preputial glands). Again, previous experiments suggest that anabolic rather than androgenic activity may be most decisive in preventing digitoxin poisoning. Be this as it may, the steroids endowed with the greatest anabolic and androgenic activity are also most potent in protecting against digitoxin.

At first sight it would seem improbable that this relationship should be merely coincidental; yet spironolactone, one of the most active digitoxin antagonists, possesses no anabolic or androgenic properties. Further research will be required to clarify the possible relationship between antidigitoxin and anabolic or androgenic activity. The possibility cannot be excluded that some steroids, for example, nandrolone phenpropionate and decanoate, would have been more potent anabolic androgenic and antidigitoxin agents had they been administered parenterally. The solvent, the number of medications per day, and many other factors may influence the efficacy of these steroids. On the basis of received data it can be seen that all steroids exhibiting great anabolic potency under the experimental circumstances employed in this research, counteract digitoxin toxicity in the rat, whereas the reverse is not true in view of the high antidigitoxin potency of spironolactone. Hence, it may be concluded that whatever the mechanism of the antidigitoxin effect, it does not necessarily depend upon anabolic activity.

Perusal of the earlier literature reveals several possibly pertinent data. Spayed female dogs, prophylactically treated with estrogens, are more resistant to the production of arrhythmia by a toxic intravenous dose of digoxin than untreated castrate controls (21). Yet, estradiol possesses no anabolic potency (14, 19) nor did it protect the rats against fatal digitoxin intoxication in the present experiments.

It has also been noted that anabolic steroids stimulate the production of hepatic microsomal enzymes which not only destroy digitalis compounds *in vitro* but also protect against the anesthetic effect of barbiturates (22, 23).

Furthermore, intact female and castrate male rats are more sensitive to the anesthetic action of progesterone than intact males, and the resistance of castrate males and females can be raised by methyltestosterone. Hence, the comparative resistance of the intact male was ascribed to a testicular compound, presumably an anabolic androgen (24).

Finally, pretreatment with various anesthetic steroids increases the resistance of the rat not only to other anesthetic steroids (25) but also to metrazol (26) and picrotoxin (27).

It would be premature to speculate upon the possible relationships between these earlier observations on the induction of resistance to various drugs by pretreatment with steroids and the present findings. However, there can be no doubt that spironolactone as well as several anabolic androgens can protect the rat against an otherwise fatal digitoxin poisoning.

REFERENCES

(1) H. Selye, *Canad. Med. Assoc. J.*, **47,** 515(1942).

(2) H. Selye and E. I. Pentz, *ibid.* **49,** 264(1943).

(3) R. Gaunt, A. A. Renzi, and J. J. Chart, *J. Clin. Endocrinol. Metab.*, **15,** 621(1955).

(4) J. Genest, J. de Champlain, R. Veyrat, R. Boucher, G. Y. Tremblay, C. G. Strong, E. Koiw, and J. Marc-Aurele, *Circulation Res.*, **17** (1965); Suppl. *Hypertension*, **13,** 97.

(5) J. H. Largh, *Am. J. Med.*, **21,** 423(1956).

(6) J. A. Luetscher, Jr., A. Dowdy, J. Harvey, R. Neher, and A. Wettstein, *J. Biol. Chem.*, **217,** 505(1955).

(7) J. A. Luetscher, Jr., R. Neher, and A. Wettstein, *Experientia*, **12,** 22(1956).

(8) C. M. Kagawa, "Hormonal Steroids," vol. 1, Academic Press, New York, N. Y., London, England, 1964, p. 445.

(9) C. M. Kagawa, In. F. M. Sturtevant, and C. G. van Arman, *J. Pharmacol. Exptl. Therap.*, **125,** 123(1959).

(10) H. Selye, "The Pluricausal Cardiopathies," Charles C Thomas, Springfield, Ill., 1961, p. 438.

(11) H. Selye, S. Grasso, and N. Padmanabhan, *Lancet*, Dec. 17, 1351(1960).

(12) H. Selye, M. Krajny, and L. Savoie, *Science*. **164,** 842(1969).

(13) H. Selye, I. Mécs, and L. Savoie, *Anesthesiology*, in press.

(14) H. Selye, *Acta Endocrinol., Kbh.*, **25,** 83(1957).

(15) H. Selye, R. Strebel, and L. Mikulaj, *J. Am. Geriat. Soc.*, **11,** 1(1963).

(16) H. Selye, B. Tuchweber, and G. Gabbiani, *ibid.*, **12,** 207 (1964).

(17) B. Tuchweber, G. Gabbiani, and H. Selye, *Am. J. Clin. Nutr.*, **13,** 238(1963).

(18) H. Selye and S. Renaud, *Am. J. Med. Sci.*, **235,** 1(1958).

(19) H. Selye, B. Tuchweber, and M. Jacqmin, *Acta Endocrinol. Kbh.*, **49,** 589(1965).

(20) K. Kovács and A. Somogyi, *Proc. Soc. Exptl. Biol. Med.*, in press.

(21) E. H. Grinnell, J. R. Johnson, J. R. Rhone, A. Tillotson, J. Noffsinger, and M. N. Huffman, *Nature*, **190,** 1117(1961).

(22) J. Booth and J. R. Gillette, *J. Pharmacol. Exptl. Therap.*, **137,** 374(1962).

(23) G. P. Quinn, J. Axelrod, and B. B. Brodie, *Biochem. Pharmacol.*, **1,** 152(1958).

(24) H. Winter, *Endocrinology*, **29,** 790(1941).

(25) H. Selye, *J. Immunol.*, **41,** 259(1941).

(26) H. Selye, *J. Lab. Clin. Med.*, **27,** 1051(1942).

(27) E. L. Clarke, *Proc. Can. Physiol. Soc. Montebello Meeting*, Oct. 24–25, 1941.

ACKNOWLEDGMENTS AND ADDRESSES

Received April 15, 1969 from *Institute of Experimental Medicine and Surgery, University of Montreal, Montreal, Canada*

Accepted for publication June 3, 1969.

This work was supported by the Ministère de la Santé, Québec, and the Medical Research Council of Canada (Block Term Grant MT-1829).

The authors also wish to thank all the companies mentioned in the section *Materials and Methods* for their contribution of the drugs used.